Utilization of a New Toxicity Testing System As a Water Security Monitoring Tool

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Introduction:

The events of September 11th have brought the potential vulnerability of critical US infrastructure to the Nation's attention. A prime target for terrorist assault is the Nation's drinking water systems. An attack aimed at drinking water distribution systems could result in numerous casualties and cripple the population's general psyche. Bogus news reports from a radio show reporting that the local water supply had been contaminated with Dihydrogen Monoxide (Water) caused a near panic in one Midwestern community.[1] That water systems are considered a viable target by al-Qaida and other terrorist organizations has been amply demonstrated by the arrest of a group of men in Rome, Italy earlier this year. The suspected terrorists were found to be in possession of potassium ferricyanide and maps of the water distribution system around the US embassy.[2]

Because of these vulnerabilities Drinking Water Utilities, Public Health Professionals, and First Responders have voiced interest in the need for an inexpensive easy-to-use, quick method to evaluate drinking water suspected of contamination. Already, drinking water professionals are instituting more elaborate and comprehensive laboratory analysis when ratepayers notify utilities about water quality concerns. Public inquiries about water quality are no longer managed as routine consumer complaints. Increased surveillance of finished drinking water increases this need for quickly deployable and easy-to-use test methods. This paper explores a possible method to detect tampering with the water supplies using an inexpensive test kit system.

Toxicity Testing as a Water Surveillance Tool:

The vast number of chemical agents that could be utilized by a terrorist to compromise a water supply system precludes monitoring on an individual chemical basis. Chemical warfare agents such as VX, Sarin, Soman, etc.; commercially available herbicides, pesticides and rodenticides; street drugs such as LSD and heroin; heavy metals; radionuclides; cyanide and a host of other industrial chemicals could be exploited as weapons.[3] This suggests utilizing some general form of toxicity testing to monitor for changes in water integrity.[4]

Toxicity is the ability of a chemical or mixture of chemicals to cause a living organism to undergo adverse effects upon exposure. These effects can include negative impacts on survival, growth, reproduction, etc. Toxicity tests are analytical experiments which attempt to detect or quantify toxicity in a sample by measuring the results exposure produces on standard test organisms.

Toxicity testing has a long history of use. The food testers employed by Roman Emperors and medieval kings were a rudimentary form of toxicity testing. The probability of getting someone to volunteer for this sort of work today is not high, and even if volunteers were available, OSHA would most likely object. This presents a problem with toxicity testing for threats to human health. No other organism will respond to a toxin in exactly the same way as a human.

The closer an organism is to humans on the evolutionary tree, the closer its responses to toxins should mirror human responses. This is why clinical toxicologists have long used other mammals such as monkeys, dogs, rats and mice in their toxicological studies.

The expense and slow response time involved with using these types of organisms to monitor water supplies is prohibitive for general applications. The next best alternative would be to use lower order vertebrates, such as fish, as surrogates. On-line monitoring of captive populations of fish, such as trout, has been utilized for several years, most notably in Europe, to evaluate source water for contamination before it enters treatment plants.[5] This is an expensive and time-consuming arrangement. While technologically simple, the maintenance of fish stocks and the interpretation of changes in fish action or viability can be difficult. Interactions with parameters such as water temperature and turbidity can skew results. Also, the chlorine present after treatment would damage the fish's gill structure and would make such a system useless for monitoring in the distribution system. This type of system has limited applications and would be of most use in larger systems for monitoring surface water feed supplies.

Another option is to use invertebrates. These organisms are even further removed in their equivalence to human responses, but their ease of use and lower maintenance costs make them a viable choice. Instruments using organisms such as the water flea, *Daphnia*, have been developed and are in use.[6] They tend to suffer from some of the same problems as fish based systems, and the instruments themselves tend to be expensive.

This leaves microorganisms, such as bacteria, as the last viable alternative. While they are far removed from humans and would be expected to exhibit differential responses from humans, their simplicity, low cost, and ease of use make them the organisms of choice.

Bacterial Monitoring of Toxicity

The concept of using microorganisms to monitor toxicity has been used for many years in the wastewater industry as a means to safeguard the bacterial populations in treatment systems from shut down due to toxic exposure. A wide variety of testing procedures and methods have been developed to monitor the toxic characteristics of influent to treatment plants. One type of commercially available test functions by measuring the effect of toxins on the light output of luminescent bacteria. This test often correlates well with total toxicity, but it does have some limitations. Bioluminescence is not an essential metabolic pathway nor is it widespread among living organisms. Toxins that specifically inhibit luciferase, the enzyme responsible for bioluminescence, may not exhibit general toxicity to other organisms. In addition, the measurement of bacterial luminescence requires the use of very expensive instrumentation

Another type of commercially available test measures the cleavage of X-Gal or ONPG by the β-galactosidase enzyme of a strain of *Esherichia coli*, which is highly sensitive to toxins. What is actually being measured in this test is the ability of one specific strain of bacteria to synthesize the enzyme β-galactosidase. The test is limited to one genetically engineered strain of *Esherichia coli* that may or may not be representative of general toxicity.

A third type of test is also commercially available. It measures the reduction of oxygen consumption by bacteria. Oxygen consumption is a good measurement of overall bacterial health, however this method does present some problems. Drawbacks are that the test requires the use of a dissolved oxygen electrode, an expensive piece of equipment. The samples must be aerated for 30 minutes before staring the test. This aeration could result in the loss of volatile toxins from the sample. Finally, only one test can be run at a time; multiple tests require long periods of time coupled with extensive sample manipulation.

The above tests are designed to be used with a specific organism. Users are not given the option of determining the toxicity of substances to other microbes or one indigenous to their own system. The inability of the other methods to utilize the normal flora contained in a given system along with costs per test is a major concern that is addressed by the proposed method described here.

Chemistry of the Proposed Method Explained

Liu *et. al.,* in an article entitled “A Rapid Biochemical Test For Measuring Chemical Toxicity” (Bulletin of Environmental Contamination and Toxicology., 26, 140-149 (1981)), described a biochemical toxicity test based on the reduction of resazurin by bacterial respiration. Resazurin is a redox-active dye which, when reduced, changes color from blue to pink. Resazurin is in the oxidized, blue, state at the beginning of the test. The bacteria oxidize the glucose added to the sample with the dye and reduce the resazurin. First, the resazurin is reduced by 2 electrons to resorufin, which has a pink color. Resorufin can be further reduced by two more electrons to dihydroresorufin, which is colorless. Dihydroresorufin can be reoxidized by atmospheric oxygen to resorufin. It is important that readings be taken before a significant amount of resorufin has been reduced to prevent interference. If the reaction time is too long, the indicator is too far reduced and interference will result. Substances that are toxic to bacteria can inhibit their metabolism and thus, inhibit the rate of resazurin reduction. This inhibition of resazurin reduction is taken as an indication of toxicity in the test. The Liu method involves an organic solvent extraction and centrifugation; these steps are time consuming and cumbersome. Also, the extracting solvent used, n-amyl alcohol, has toxic properties which may affect test results.

The method proposed in this paper follows the same concept as Liu’s method but has been modified to alleviate some of its drawbacks. The proposed method makes use of an accelerant (gluteraldehyde:: U.S. patent 5,413,916 assigned to Hach Co.) in the method to reduce the reaction time . This prevents oxygen interference and also, allows the use of a lower level of inoculum to reduce the dye. Therefore, due to the decrease in turbidity resulting from a smaller inoculum, the absorbance of the dye can be read in a colorimeter or spectrophotometer without removing the bacterial cells from the light path. While the exact mode of action that allows gluteraldehyde to speed up the process of respiration is unknown, it is most likely the result of the uncoupling of oxidation from phosphorylation during respiration.

Respiration is the process that generates ATP (adenosine triphosphate) using the energy from the oxidation of an electron donor by an external electron acceptor. In the case of aerobic respiration the terminal electron acceptor is oxygen (O_2). Electrons are transferred from the electron donor to the terminal electron acceptor (resazurin in the test) through a series of electron-transfer proteins that are embedded in the cell membrane. Substances, such as redox reactive dyes like resazurin, can act as alternative electron acceptors by oxidizing one of the membrane proteins.

Electron transfer is coupled to ATP generation by a proton gradient across the cell membrane that is created as electrons flow through the membrane bound electron transfer system. ATP is synthesized from ADP (adenosine diphosphate) and inorganic phosphate when this proton gradient is used to drive the phosphorylation of ADP by a membrane spanning ATPase.

Since it involves the creation and maintenance of a proton gradient across the cell membrane, respiration is affected by substances that make the membrane permeable to protons. When protons can cross the membrane freely, bacteria cannot use a proton gradient to make ATP. The oxidation of energy sources is thereby uncoupled from the phosphorylation of ADP. Substances that uncouple oxidation from phosphorylation in this way are often organic acids like phenols that are lipid soluble but can become protonated on the outside of the membrane, diffuse across the membrane and then become deprotonated on the inside of the membrane.

Since the rate of a catabolic process is controlled by the ADP/ATP ratio, the uncoupling of oxidation from phosphorylation accelerates these processes. Some substances that cannot act as proton shuttles uncouple oxidation from phosphorylation by inhibiting the ATPase that uses the proton gradient to synthesize ATP. It is possible that gluteraldehyde acts in this way.

Gluteraldehyde has the ability to accelerate the resazurin reaction in many different species of bacteria including both Gram positive and Gram negative species. There is a possibility that it will work with some eukaryotic species, though this has not been tested. This allows the user to culture his/her own organisms and determine the toxicity of a sample to the bacteria that will be exposed to it.

Regardless of the mechanism, the result of the action of uncouplers (possibly gluteraldehyde) in the toxicity test is twofold. Reactions that are dependent upon the concentration of reduced enzymes and ATP are slowed due to a decrease in the concentration of substrates. On-the-other-hand, reactions like the reduction of resazurin that are dependent upon the rate of electron transfer are accelerated.

The use of gluteraldehyde to accelerate the reaction allows the avoidance of many of the problems associated with Liu's original method. It allows the use of a smaller number of bacteria to reduce the resazurin dye. Consequently, the absorbance of the dye can be read instrumentally without removing the bacterial cells from the light path. This alleviates the need for organic extraction and/or centrifugation. With the reduced turbidity, the color change of the dye is dramatic and can be easily distinguished visually. Also, the decreased reaction time eliminates the interference caused by over-reduction of the dye.

Thus, the proposed test can be performed rapidly compared to other tests. In addition, the proposed method allows the utilization of an inexpensive colorimetric technique without extraction, filtering, or centrifuging. The test can be used as a visual method. Finally, this test, unlike other tests, can be used with many different species of bacteria or mixed cultures.

Procedure

As with all toxicity tests using surrogate organisms, it is important to remember that this method is not all-inclusive. There are substances that are detrimental to humans that will show no response to this test. There are also substances, such as copper, that may show a dramatic response with this method and yet, represent little or no human toxicity.

1) First, the inoculum for the test must be prepared. This is done by growing a bacterial culture at 35^0C in a Lauryl Tryptose Broth Tube for 10 to 72 hours. The culture should be visibly turbid. The bacteria can be obtained from the system being monitored or standard stock cultures may be used as a source of the inoculum.

2) Next, determine the number of tests to be run; several samples from different sources or serial dilution of a single source may be run at the same time. At least one blank needs to be run at the same time as the sample(s). This blank can consist of DI water that exhibits no measurable toxicity, or another water source that has been chosen to represent zero or baseline toxicity may be used. For the monitoring of drinking water samples, it may be best to obtain a large reservoir of water that will be retained and used as a baseline from which any deviation can be charted. This will cancel out most superfluous contaminants, such as copper, that are not of interest. Sample and blank water samples should be at the same temperature. After the number of tests to be run has been determined, add 5 mL of blank water or sample to the appropriate tubes.

3) Add 1 drop of 0.0246 N sodium thiosulfate to each of the tubes, including the blank, to remove any residual chlorine. This step may be omitted if the water is not chlorinated.

4) Add 2 drops of the accelerator solution (gluteraldehyde) to each tube.

5) Then add one of the reagent pillows. These pillows contain the resazurin dye and all of the food and nutrient sources needed to support active cell respiration.

6) Add 0.5 mL of the culture prepared in step 1 to each of the tubes. The cultures should be agitated before use to ensure even dispersal of the organisms.

7) Shake vigorously to dissolve all of the powder and mix the solution. Shaking also serves to fully oxygenate the samples assuring that oxygen concentration is not a factor in determining respiration rate.

8) Measure and record the Abs for each tube at 603 nm.

9) Allow the solutions in the tubes to react until the Abs of the blank has decreased 0.600 ± 0.100. This should take 45- 75 minutes. (Exact reaction time will vary depending upon a number of factors including temperature, age of culture, number of bacteria per 0.5 ml, etc. Note that incubation of the tubes should occur at room temperature. The accelerator solution will speed the reaction at room temperature (20-25^{0}C), but it will tend to decrease the rate of reaction at 35^{0}C.)

10) After the blank has decreased in Abs 0.600 ± 0.100, measure and record the Abs for each tube.

11) Calculate the change in Abs for each tube.

$$\Delta \text{ Abs} = \text{Abs step 8} - \text{Abs step 10}$$

12) Calculate the % Inhibition for each sample.

$$\%I = \{1 - (\Delta\text{Abs sample} \div \Delta\text{Abs blank})\} \times 100\%$$

Interpreting Results

The % Inhibition results obtained are only a relative measurement and do not represent a true quantitative measurement of toxic concentration. The % Inhibition does not necessarily increase in direct proportion to the concentration of toxins. In order to determine the minimum inhibition concentration of a toxin, it is possible to make tenfold dilutions of the sample and determine the % Inhibition for the dilutions until the sample is diluted sufficiently so that no inhibition is observed. This is known as the no observed effect concentration (NOEC).

Due to the many variables involved in the test, the limits of detection are on the order of 10% Inhibition. This would correlate to the lowest observed effect concentration (LOEC). If a sample shows less than 10% Inhibition, the test should be repeated. After several repetitions it is advisable to look at the series of data to determine the likelihood of toxicity. Results below 10% are not reliable, but can be used to surmise some presence of toxicity if they are consistent. See examples below.

Table 1

Data Points: % Inhibition	Conclusion
7%, 9%, 5%, 8%, 5%	May be slightly toxic.
7%, -4%, -5%, 5%, 1%	Most likely not toxic.
-7%, -9%, -5%, -8%, -5%	May be slightly toxic.

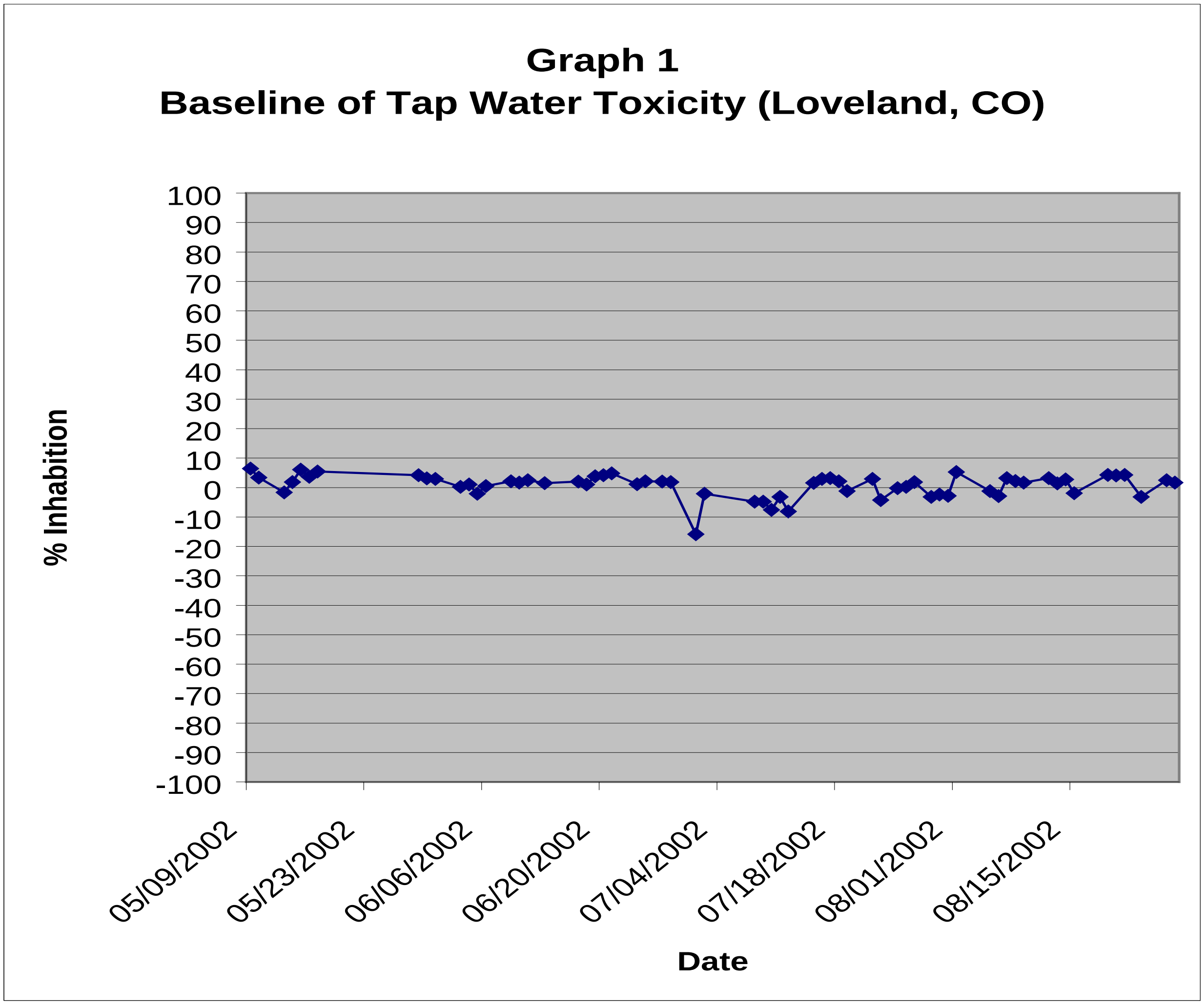
Some toxins will increase respiration and will give a negative % Inhibition on this and all other respiration-based toxicity tests. After repeated testing, samples that always give a % Inhibition that is more negative than –10% should be considered toxic. Some compounds may exhibit a change from negative to positive inhibition or vice versa as the concentration of the substance is increased. This may be due to the differing effects of the substance. For example at low concentrations it may act as a supplemental food source or nutrient while at higher levels it acts as a toxin.

Experimental Results. Baseline Study:

The first step in establishing a monitoring program for a given tap water system is to establish a baseline on water of acceptable quality. All tests can be run versus DI water as a baseline, but to center the results around zero toxicity, it may be preferable to establish a reservoir of the water to be tested and run all tests versus that source. This will compensate for any metals or other toxicants that are routinely present in the water to be monitored.

As an example, a baseline was established for the tap water at the Hach facility in Loveland, Colorado. The Day before the series was to begin, the tap water was allowed to run for 10 minutes to equilibrate and then a 5 liter fluorinated high-density polyethylene container was filled with the water to use as a set point to run all future tests against.

For the course of the study, the tap water was allowed to run for 10 minutes and then a sample was taken each day. The proposed procedure was performed using the water from the reservoir as the blank. The water temperature was allowed to equilibrate before testing. A stock culture of *E coli* derived form Aqua-QC Stiks™ was used as the innoculant. (Note: Any lyophilized bacteria culture will suffice.. Aqua-QC Stiks™ is a registered trademark of Microbiologics Inc.) Thiosulfate was added to both the samples and the blanks to ensure uniformity. All tests were done in triplicate and an average of the results was reported. See Graph 1



Investigation of Compounds of Concern for Drinking Water Security:

There are a large number of compounds that could be deployed by terrorists in an assault on water supplies. Among these are; heavy metals; commercially available herbicides, pesticides and rodenticides; street drugs such as LSD and heroin radionuclides; industrial chemicals including cyanide and chemical warfare agents such as VX, Sarin, Soman, etc.

No form of toxicity test can be expected to respond to all of these agents. In the following experiments, a number of these compounds were investigated as to their ability to elicit a response from the proposed test.

The agents being tested were dissolved in DI water and tested versus the same DI water source without agent added. All tests were run in triplicate, and the number reported is the average response. If it was necessary to add a solvent such as methanol to get a given reagent to dissolve, a like amount was added to the DI water blank so as to ensure that the response was from the agent and not due to solvent effects.

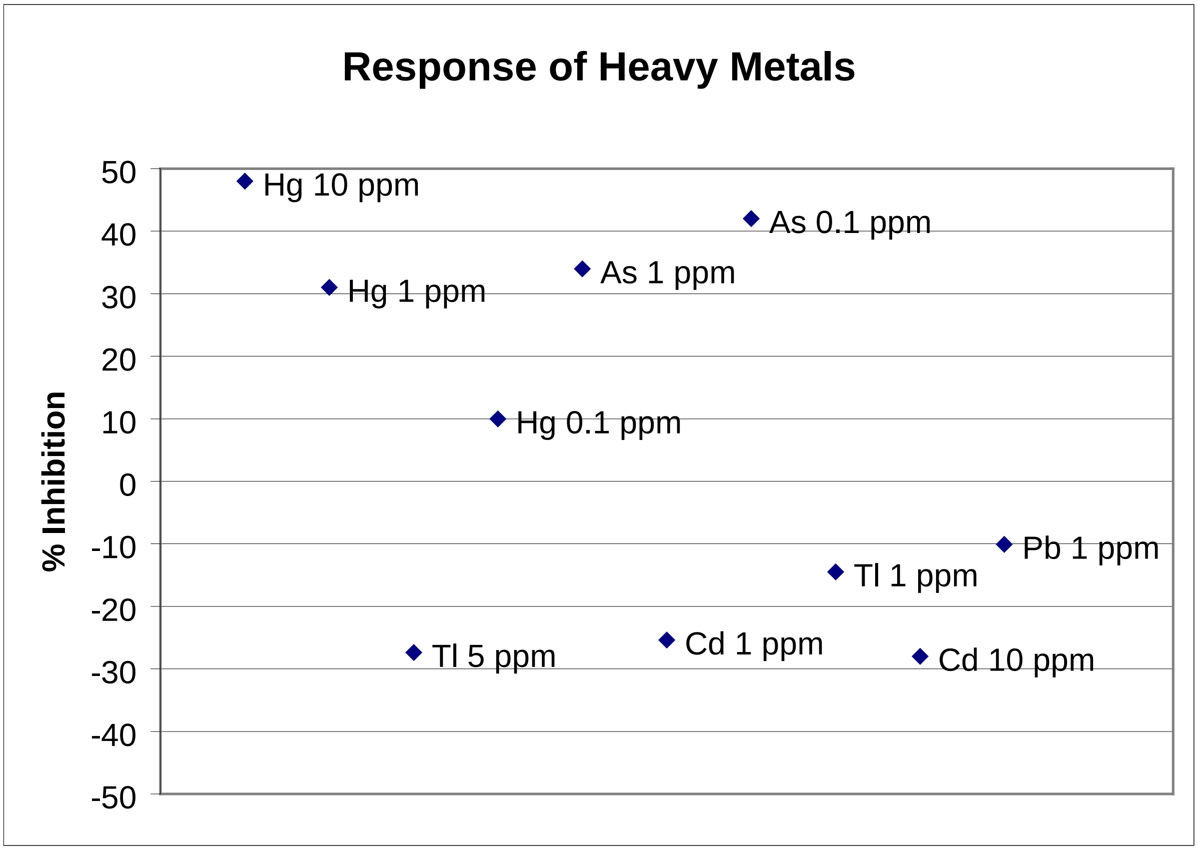
Heavy Metals:

Heavy metals are agents of concern due to their toxicity to humans. They are also fairly easy to obtain, and their salts tend to be readily soluble. A number of metals were investigated at various concentrations. See Table 2 and Graph 2.

Table 2
Response of Heavy Metals

Compound	Concentration	% Inhibition
Arsenic	1.0 ppm	34.0
Arsenic	0.1 ppm	42.0
Cadmium	10.0 ppm	-28.0
Cadmium	1.0 ppm	-25.4
Lead	10 .0 ppm	-26.5
Lead	1.0 ppm	-10.1
Mercury	10.0 ppm	48.0
Mercury	1.0 ppm	31.0
Mercury	0.1 ppm	10.0
Thallium	5.0 ppm	-27.4
Thallium	1.0 ppm	-14.5

Graph 2



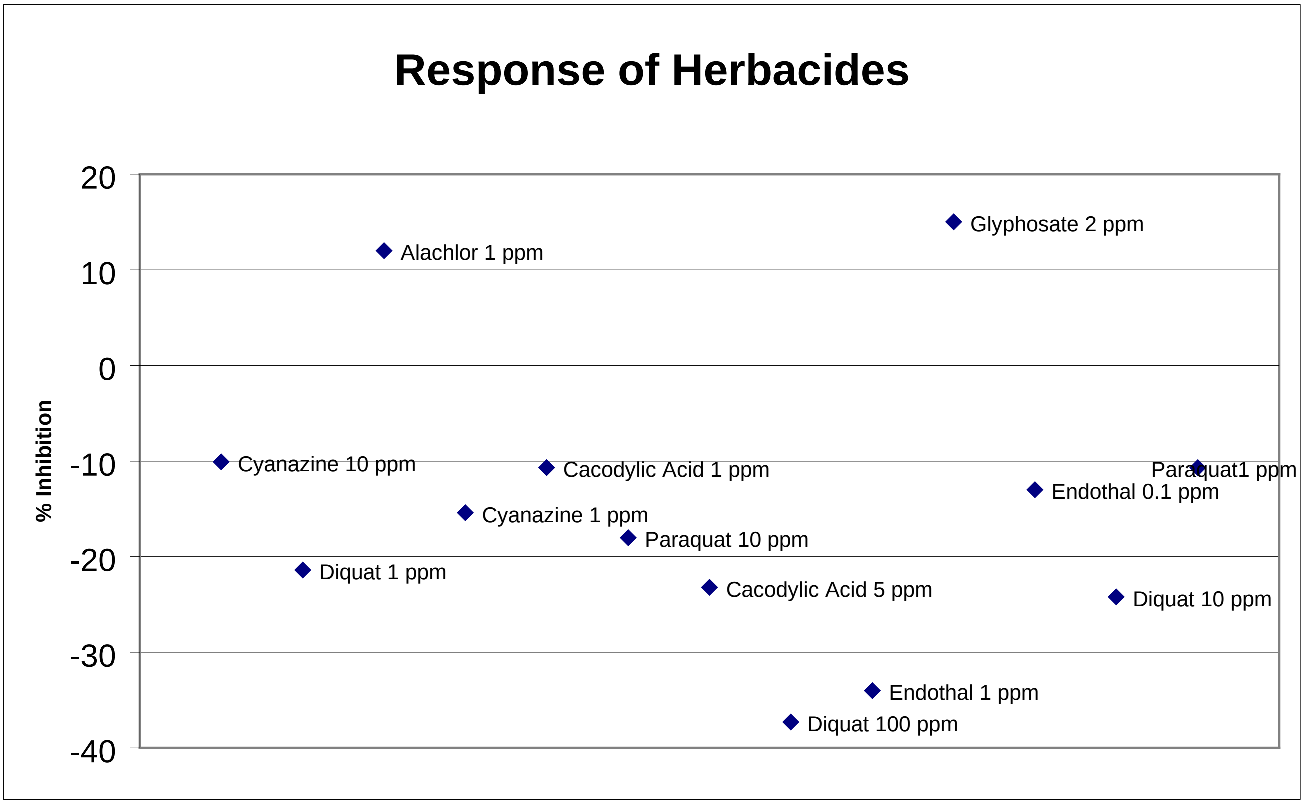
Herbicides Investigated

While as a general class, herbicides tend to be less detrimental to human health than some other compounds; there are some notable exceptions (e.g. Endothal).[7] This along with the ability to easily obtain large quantities of these chemicals from agricultural supply sources adds to the concern. Even if few fatalities resulted, the panic caused by the introduction of herbicide type compounds into a water system could be severe; therefore, a number of likely compounds were investigated. See Table 3 and Graph 3.

Table 3
Response of Herbicides

Compound	Concentration	% Inhibition
Alachlor	1.0 ppm	12.0
Cacodylic Acid	5.0 ppm	-23.2
Cacodylic Acid	1.0 ppm	-10.7
Cyanazine	10.0 ppm	-10.1
Cyanazine	1.0 ppm	-15.4
Diquat	100.0 ppm	-37.3
Diquat	10.0 ppm	-24.2
Diquat	1.0 ppm	-21.4
Endothal	1.0 ppm	-34.0
Endothal	0.1 ppm	-13.0
Glyphosate	2.0 ppm	15.0
Paraquat	10.0 ppm	-18.1
Paraquat	1.0 ppm	-10.7

Graph 3



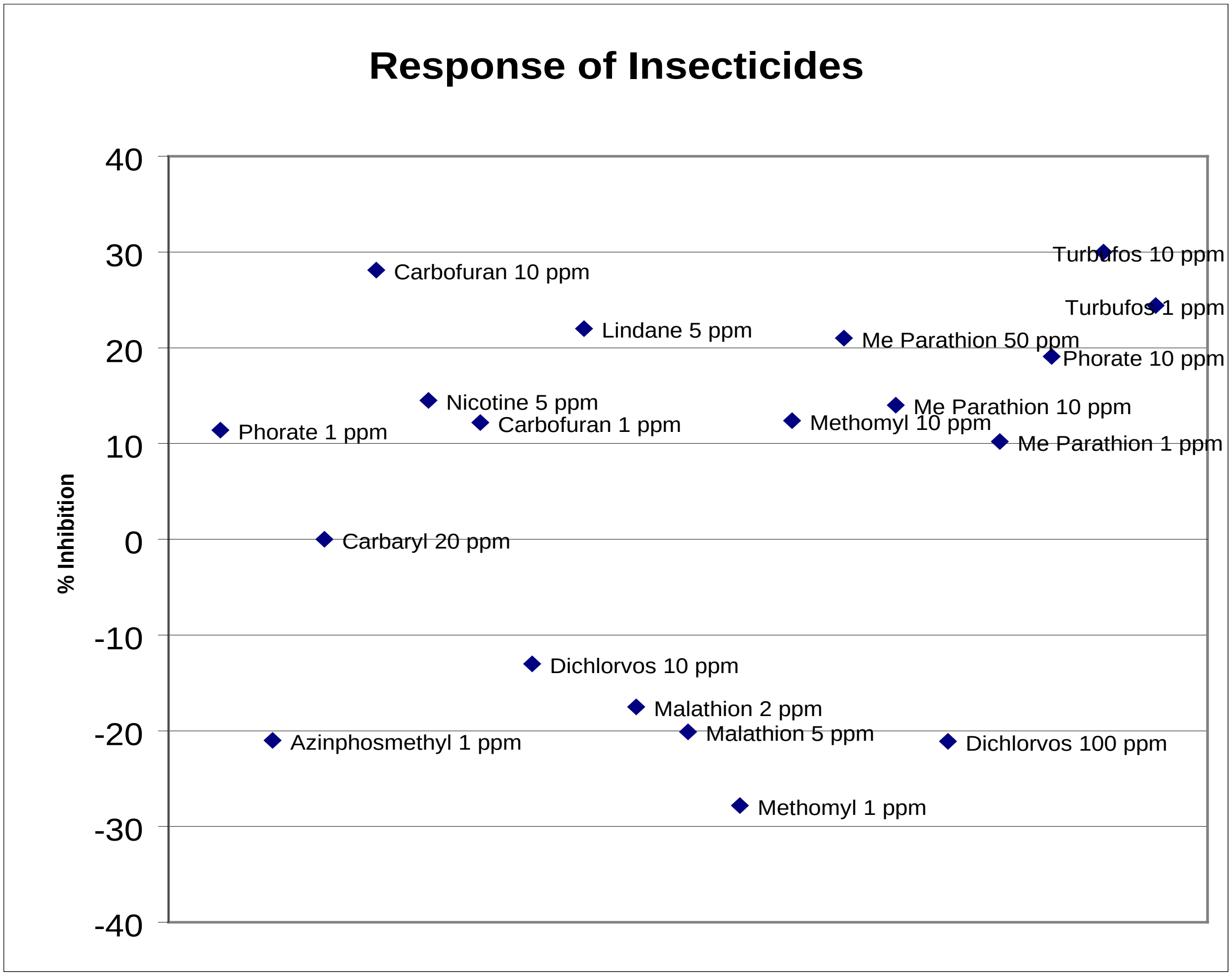
Insecticides Investigated:

Insecticides tend to be more harmful to human health than herbicides. Some of the insecticides have chemical structures quite similar to some of the chemical warfare nerve agents, and there are several that are cholinesterase inhibitors.[7] Like herbicides, insecticides are also readily available in large quantities. For some, their solubility limits their usefulness as water introduced weapons, but others are quite soluble and present more of a threat. A number of these compounds were investigated. See Table 4 and Graph 4.

Table 4
Response of Insecticides

Compound	**Concentration**	**% Inhibition**
Azinphosmethyl	1.0 ppm	-21.0
Carbaryl	20.0 ppm	No response
Carbofuran	10.0 ppm	28.1
Carbofuran	1.0 ppm	12.2
Dichlorvos	100.0 ppm	-21.1
Dichlorvos	10.0 ppm	-13.0
Lindane	5.0 ppm	22.0
Malathion	2.0 ppm	-17.5
Malathion	5.0 ppm	-20.1
Methomyl	1.0ppm	-27.8
Methomyl	10 .0 ppm	12.4
Methyl Parathion	50.0 ppm	21.0
Methyl parathion	10.0 ppm	14.0
Methyl Parathion	1.0 ppm	10.2
Nicotine	5.0 ppm	14.5
Phorate	10.0 ppm	19.1
Phorate	1.0 ppm	11.4
Turbufos	10.0 ppm	30.0
Turbufos	1.0 ppm	24.4

Graph 4



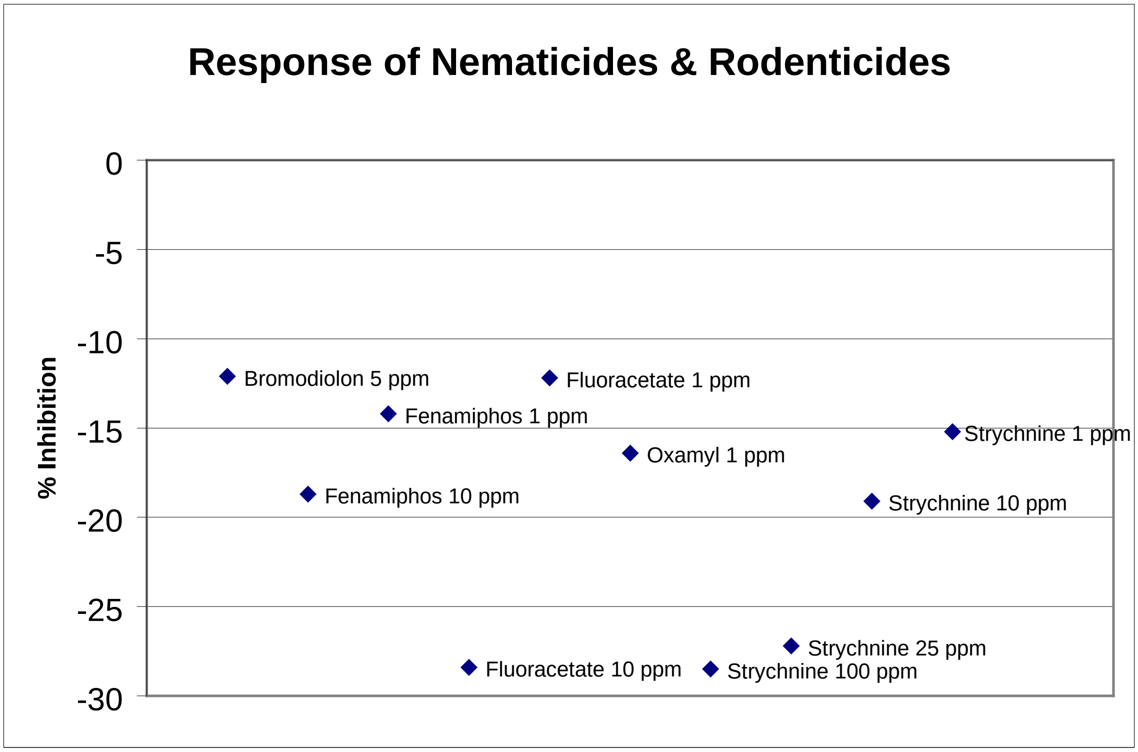
Nematocides and Rodenticides Investigated:

Nematocides are similar to insecticides. They, with some exceptions, do tend to be more soluble than insecticides. Some nematocide compounds are also similar to chemical warfare agents in structure and mode of action. Rodenticides are of concern because they are specifically designed to be lethal to mammalian species such as humans. Both classes are readily available in large quantities. A number of these compounds were investigated. See Table 5 and Graph 5.

Table 5
Response of Nematocides and Rodenticides

Compound	Concentration	% Inhibition
Bromodiolon	5.0 ppm	-12.1
Fenamiphos	10.0 ppm	-18.7
Fenamiphos	1.0 ppm	-14.2
Fluoracetate	10.0 ppm	-28.4
Fluoracetate	1.0 ppm	-12.2
Oxamyl	1.0 ppm	-16.4
Strychnine	100.0 ppm	-28.5
Strychnine	25.0 ppm	-27.2
Strychnine	10.0 ppm	-19.1
Strychnine	5.0 ppm	-15.2

Graph 5



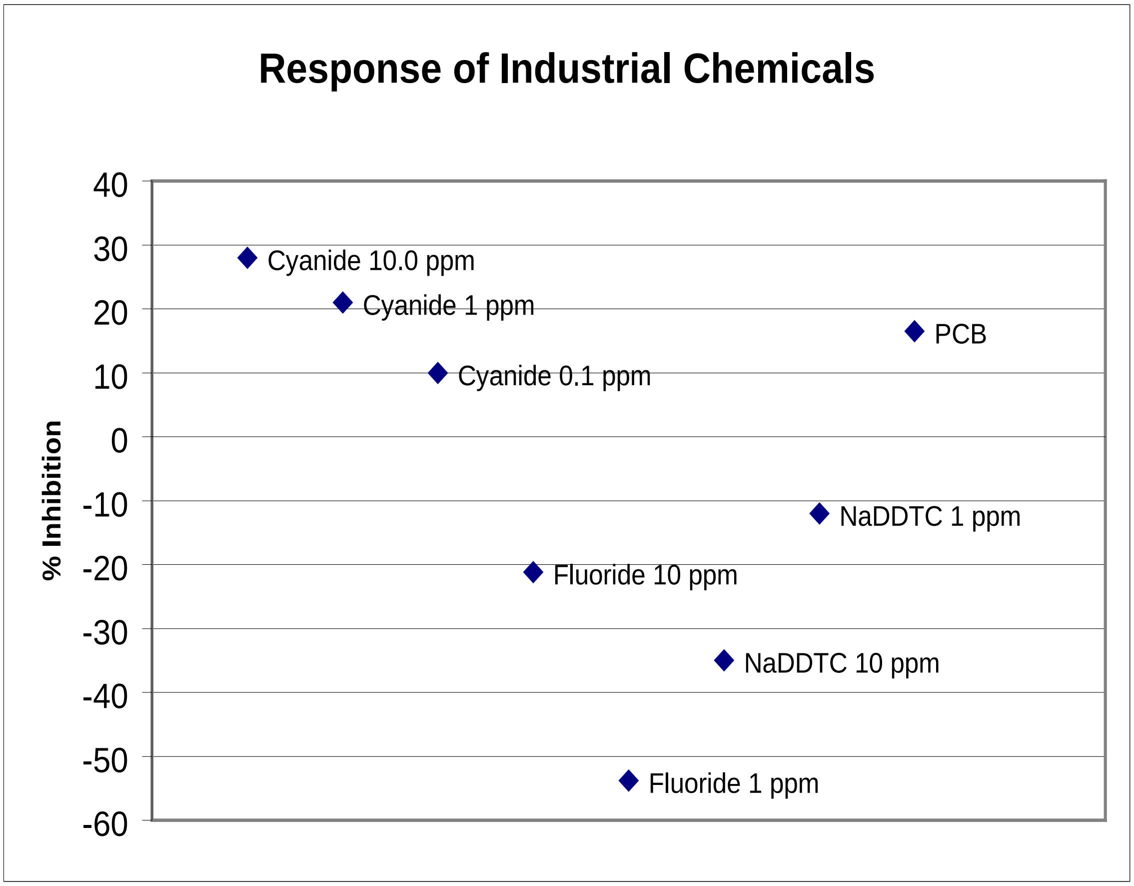
Industrial Chemicals and Miscellaneous Agents:

There are any number of industrial chemicals that could be used in an attack. Chief among these is cyanide, which is widely used in mining and other industries. That terrorists are thinking along these lines is borne out by the fact that the attempted terrorist act in Italy involved a cyanide salt.[2] While the compound in question was of limited toxicity, the mere fact that it had cyanide in its name probably led the terrorists to attempt to use it. Other industrial and miscellaneous chemicals could also be used. Several were investigated. See Table 6 And Graph 6.

Table 6
Response of Industrial and Miscellaneous Agents

Compound	Concentration	% Inhibition
Cyanide	10.0 ppm	28.0
Cyanide	1.0 ppm	21.0
Cyanide	0.1 ppm	10.0
Fluoride	10.0 ppm	-21.2
Fluoride	1.0 ppm	-53.8
Sodium Diethyl Dithiocarbamate	10.0 ppm	-35.0
Sodium Diethyl Dithiocarbamate	1.0 ppm	-12.0
PCB (Arochlor 1248)	20 ppb	16.5

Table 6



Illegal Drugs:

Illegal drugs are not widely recognized as a potential threat. Street drugs, such as LSD, GHB, PCP and heroin, among others, are a mode of attack that could be employed. Some drugs, such as LSD, are easily synthesized in a home lab[8] and exhibit human toxicity in doses that are only matched by some of the militarized nerve agents.[9] Other drugs, such as heroin, are widely available. While the cost could be prohibitive for individuals working alone, supplies do exist for well-organized and funded groups.

Also, it should be noted that a large portion of the illegal opiates (such as heroin) finding their way into the US come from areas such as West Asia where the terrorist cells are at their most active. Some of the money funding the terrorist groups may come from the illegal drug trade.[10] The Islamic fundamentalist groups are also vehemently opposed to the use of such drugs, and their rampant use in the US is one of the factors they point out when expounding on the moral decay of the Godless west. It may intrigue the terrorists to use a symbol of western moral depravity against us. The effects of LSD, a representative of the psychedelic drug family, and Heroin, one of the opiates, were investigated. See Table 7 and Graph 7

STUDY PENDING APPROVAL OF FACILLATY BY DEA

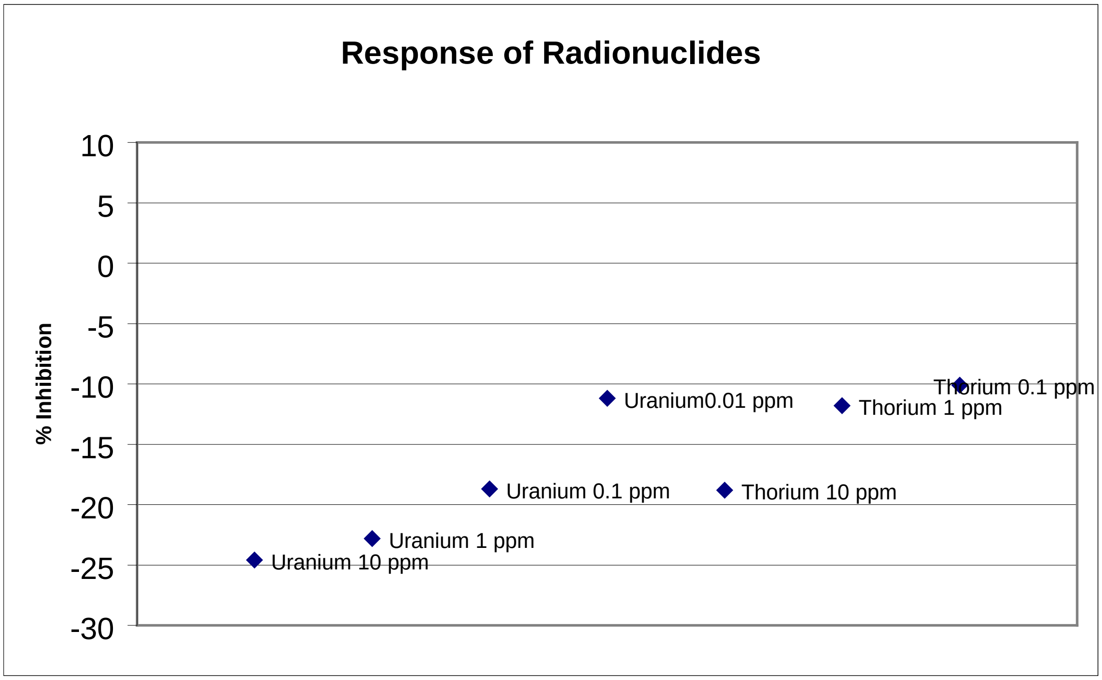
Radionuclides:

The use of radionuclides as a terror weapon is a distinct possibility. Even if casualties were low, the psychological aspect of a nuclear threat could be severe. Obtaining high purity, highly radioactive material, such as plutonium or Uranium 238, is difficult, and it is unlikely that a terrorist organization that had obtained these materials would be inclined to use them in an attack on a water system. More likely is the use of low level radioactive material or waste. Low level radioactive salts of Uranium and Thorium were studied. See table 8 and Graph 8.

Table 8
Response of Radionuclides

Compound	Concentration	% Inhibition
Uranium	10.0 ppm	-24.6
Uranium	1.0 ppm	-22.8
Uranium	0.1 ppm	-18.7
Uranium	0.01 ppm	-11.2
Thorium	10.0 ppm	-18.8
Thorium	1.0 ppm	-11.8
Thorium	0.1 ppm	-10.1

Graph 8



Commercial Products:

While not the weapons of choice for organized terrorists, lone saboteurs, the emotionally unstable or small groups may turn to easily obtained commercial products such as bug sprays or lawn chemicals. Many of the active ingredients of these preparations are the same as the pesticides and herbicides already discussed. The difference lies in the smaller proportion of active ingredients. The vast majority of these compounds have inert ingredients listed as their main component. Also, many of these compounds contain a mixture of different active ingredients. These factors may lead to a different toxicity profile from the pure ingredients. A few are evaluated in Table 9 and Graph 9.

Table 9
Response of Commercial Products

Compound	Concentration	% Inhibition
Roundup[1]	100 ppm	12.8
Roundup[1]	10 ppm	-5.4
Ortho Wasp & Hornet Spray[2]	1000 ppm	13
Ortho Wasp & Hornet Spray[2]	100 ppm	11
Ortho Wasp & Hornet Spray[2]	10 ppm	-20.1
RealKill Multipurpose Lawn & Garden Insect Killer (Dursban)[3]	1000 ppm	22.9
RealKill Multipurpose Lawn & Garden Insect Killer (Dursban)[3]	100 ppm	9.3
RealKill Multipurpose Lawn & Garden Insect Killer (Dursban)[3]	10 ppm	-28
Hot Shot Spider Killer[4]	1000 ppm	8.7
Hot Shot Spider Killer[4]	100 ppm	5.0
Hot Shot Spider Killer[4]	10 ppm	-36.7
Triacide (Diazinon)[5]	1000 ppm	-13.4
Triacide (Diazinon)[5]	100 ppm	-17.7
Triacide (Diazinon)[5]	10 ppm	-43.2

1) Roundup is 50.2% Glyphosate and 49.8 % Inert ingredients

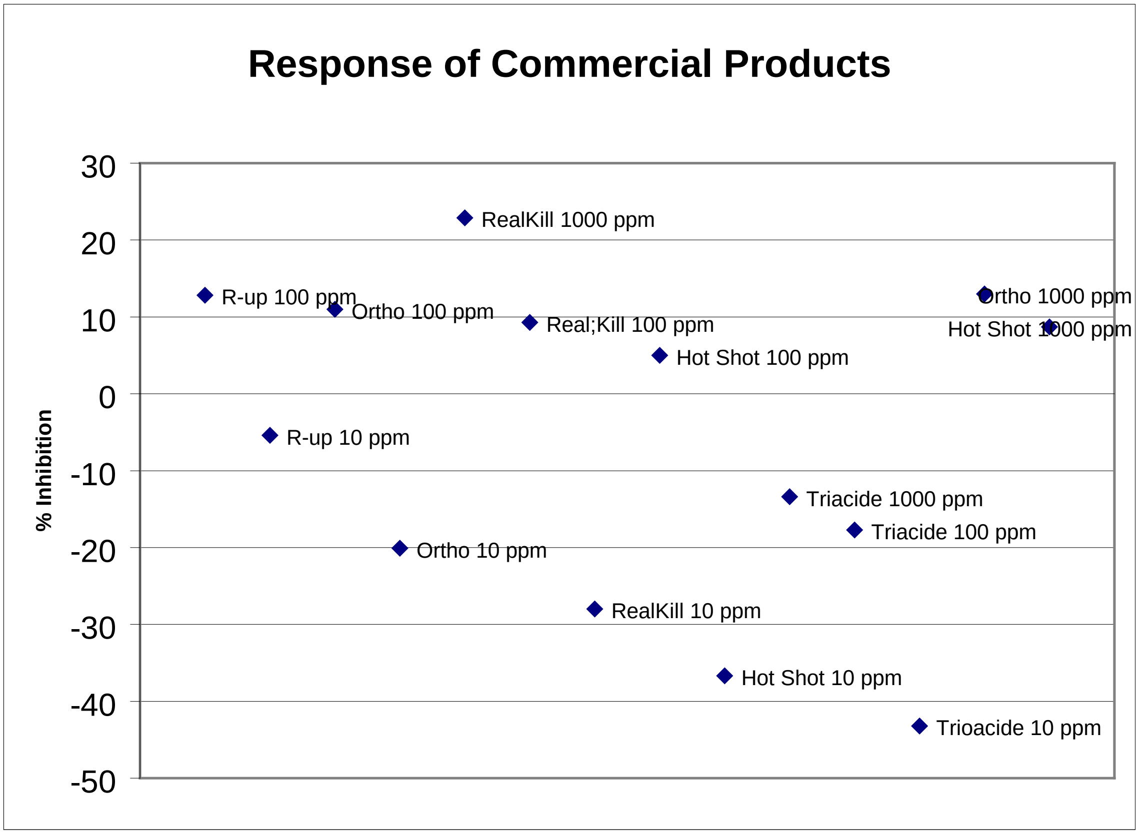
2) Ortho Wasp & Hornet Spray is 0.2% Tetramethrin, 0.2% Sumithrin and 99.6% Inert Ingredients

3) RealKill Multipurpose Lawn and Garden Insect Killer or Dursban is 2.5% Permithrin and 97.5% Inert Ingredients

4) Hot Shot Spider Killer is 0.03% Tralomethrin, 0.05% d-Trans Allethrin and 99.92% Inert ingredients

5) Triacide or Diazinon is 0.5% Lambda-Cyhalothin and 99.5% Inert Ingredients

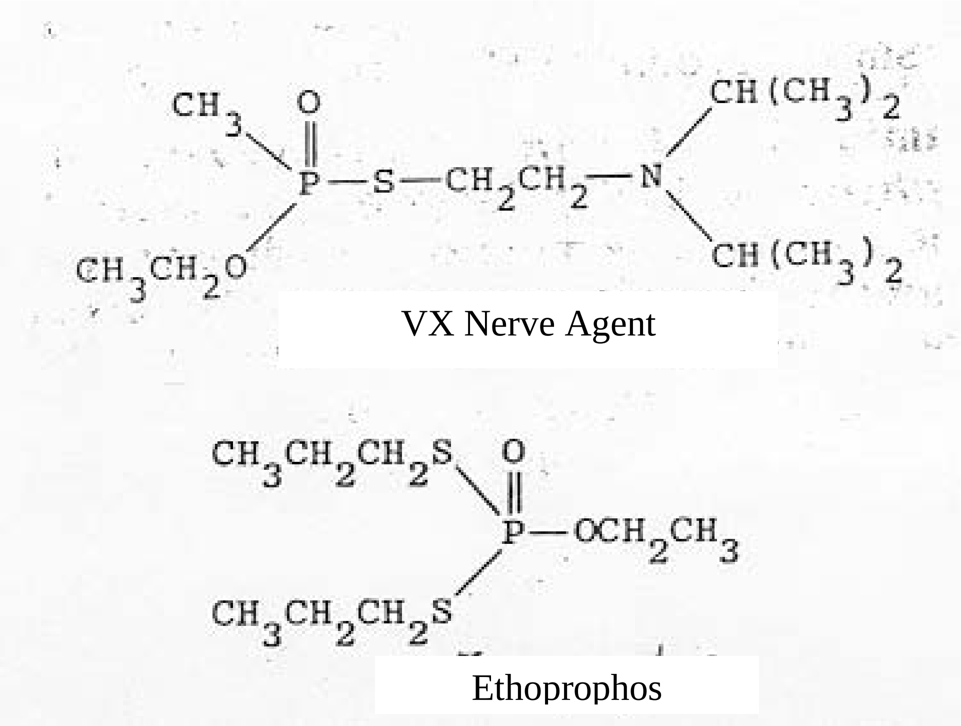
Graph 9



Chemical Warfare Agents:

Chemical warfare agents such as VX, Soman, T-2 Toxin along with older type chemical weapons such as Mustard Gas and Lewisite are not likely to be targeted against a water system. It is more likely that any assault from these weapons will be via aerosol. As the result of an aerosol attack it is possible and even likely that these agents could find their way into water supplies. Most warfare agents were not available for testing. Aflatoxin was tested along with Ethoprophos a surrogate for VX nerve agent. See Figure 1. See Table 10 and Graph 10.

Figure 1

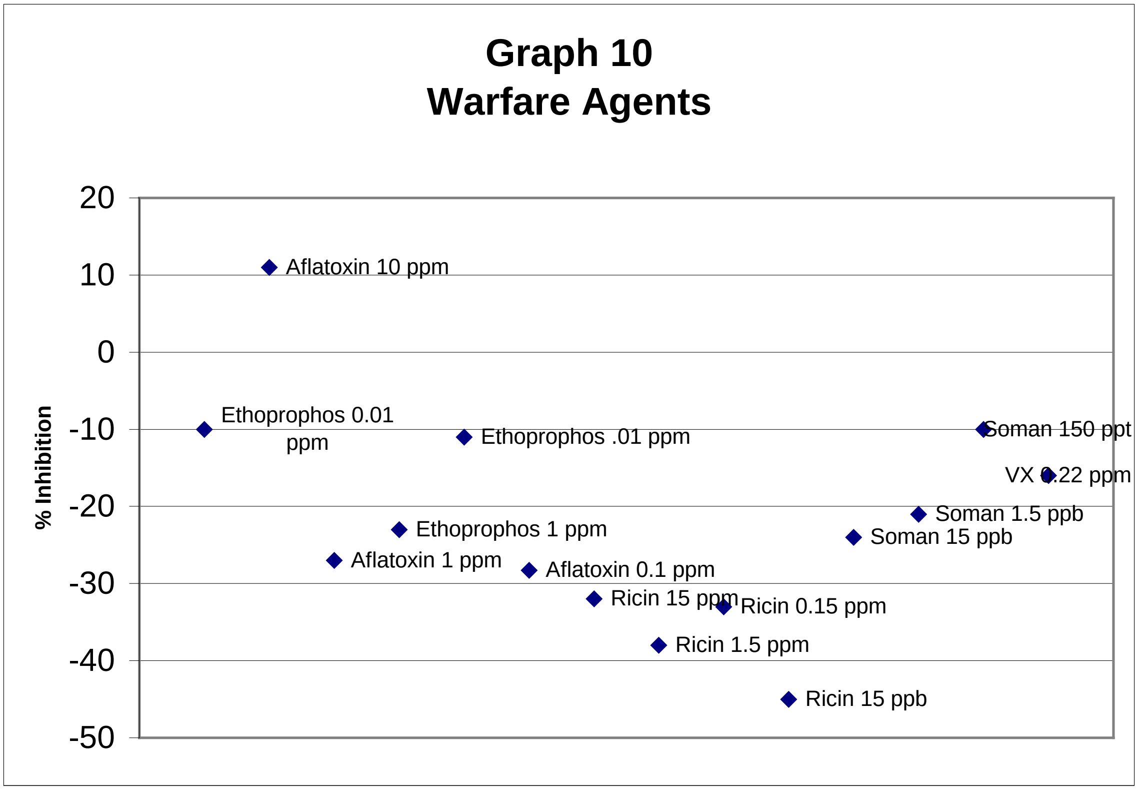


VX Nerve Agent

Ethoprophos

Table 10
Response of Warfare Agents

Compound	Concentration	% Inhibition
β Aflatoxin	10 ppm	11.0
β Aflatoxin	1 ppm	-27.0
β Aflatoxin	0.1 ppm	-28.3
Ethoprophos	1 ppm	-23.0
Ethoprophos	0.1 ppm	-11.0
Ethoprophos	0.01 ppm	-10.0
Ricin[11]	15 ppm	-32.0
Ricin[11]	1.5 ppm	-38.0
Ricin[11]	0.15 ppm	-33.0
Ricin[11]	0.015 ppm	-45.0
Soman[11]	0.015 ppm	-24.0
Soman[11]	0.0015 ppm	-21.0
Soman[11]	0.00015 ppm	-10.0
VX[11]	0.22 ppm	-16.0


Graph 10
Warfare Agents

Discussion of Results:

The purpose of running a baseline study was to determine if the baseline would hold steady enough to allow recognition of significant events in the water system. As a whole, the test preformed very well in the baseline study. Except for one anomalous data point, the range held between 6.4 and -8.1% inhibition. This is well within the ± 10% expected for the test. A reformulation of the reagent powder is undergoing study and will hopefully cut down on this variability even further.

There was one anomalous data point (<-15 % Inhibition). This appeared to be some sort of significant change. As there were no reports of illness in the area, it can be concluded that it was most likely not a serious contamination event.

A possible explanation for the sudden change in toxicity could be due to the massive forest fires in the area at the time. Throughout most of the monitoring period, the area was hazy from the smoke, but on the day in question the blanket of smoke was extremely heavy. In fact it appeared as a moderate fog and the odor could be distinctly detected. This was the only day during the monitoring period that the smoke was that heavy and may offer an explanation for the spike in toxicity.

An analysis of possible agents that a terrorist organization could use was done, and a list was compiled. As many of these reagents as possible were obtained and analyzed for their response to the proposed toxicity test. The vast majority of the reagents showed a response to the test. Some of the heavy metals exhibited a positive effect on the toxicity test (mercury and arsenic) while the others (Cadmium, Lead and Thallium) showed negative toxicity. Both positive (respiration has sped up) and negative (respiration has slowed) results greater than 10% or less than –10% are considered toxic. All of the levels tested showed a response greater than the noise in the test at levels far below what would be expected to cause acute human toxicity. The test appears to be a very good indicator of contamination by heavy metals.

It must be kept in mind however that false positives are possible. Copper, at levels as low as 2 mg/L, has been shown to exhibit toxicity levels in the test as high as 70%. That is why it is important to run the test versus a baseline reservoir that contains levels of metals commonly found in the system.

The herbicides that were tested, as was the case with the heavy metals, exhibited a wide range of responses exhibiting both positive and negative inhibition. The test was able to detect all of the herbicides screened at the stated levels. One result that appears strange is that Cyanazine exhibits a greater response at lower doses than it does at higher doses. This discrepancy is within the noise level of the test, but may also be explained, if at higher levels, the bacteria were able to begin using the compound as a carbon source, which could help to counteract some of its toxic effects during the short time frame of the test. Another factor worth noting is that the response to Endothal, which is one of the herbicides with the largest known toxic effects, occurred at very low levels and was up to - 34% at only 1 ppm.

The insecticides also showed a variety of responses with both positive and negative inhibition being exhibited. Positive inhibition seemed to be more prevalent. The test showed a response to all of the insecticides investigated except for Carbaryl. Carbaryl is fairly difficult to dissolve in water making it at unlikely candidate for use as a weapon against water supplies. To get it into solution it was necessary to place it in an ultrasonic bath for several hours. The heat generated in this process may have resulted in chemical changes rendering it less toxic. On-the -other-hand, *E. coli* bacteria may not respond to this toxin and the non-response of the test may be valid. There are definitely some substances that will not elicit a response from this test whether Carbaryl is one of them or not.

Another interesting response found in the insecticide data is exhibited by Methomyl. This compound exhibited strong negative inhibition at -27.8% at a concentration of 1 mg/L and then switched to positive inhibition of 12.4% when the concentration increased to 10 mg/L. This may be due to competing modes of toxicity or to the use of the compound by the bacteria at a low concentration as a carbon source, which is overwhelmed by its ability to slow respiration as the concentration increases. There should theoretically be a point somewhere between 1 and 10 mg/l where the toxicity is equal to zero. The chances of an event hitting the exact concentration where the test would be unable to detect it are slim.

All of the nematocides and rodenticides investigated gave a response to the test. The responses for this class all showed a negative inhibition. Common poisons like strychnine and fluoracetate were capable of being detected at levels as low as 1 mg/L.

The industrial and miscellaneous chemicals also all showed a response. The responses exhibited both positive and negative inhibition. Cyanide, an industrial chemical of prime concern due to its availability, toxicity and easy solubility, was detectable down to 0.1 mg/l. Another response of note was that of fluoride. It had a large response at as little as 1 mg/L. Fluoride is commonly added to drinking water. This helps to show the importance of using a reservoir for the baseline.

Illegal Drug Section Pending

Radionuclides are not readily available; therefore the compounds tested were radioactive but not extremely so. All of the compounds tested exhibited negative inhibition. It is not certain whether or not the exhibited toxicity was due to the radioactive nature of the materials or due to their action as heavy metals. The test however, did show good sensitivity to both Thorium and Uranium. Thorium was detectable down to 0.1 mg/L and Uranium responded down to 0.01 mg/L

The commercial products tested gave mixed results. All showed up as toxic at some level. The responses were complicated due to the fact that the majority of these compounds makeup consists of inert ingredients. These inert ingredients are not necessarily inert to the test. Also, some of the products have more than one active ingredient that may exhibit competing modes of toxicity. One component may be driving the inhibition negative while the other drives it positive. The inert ingredient may also act as a toxicant or as a food source further complicating the response. Regardless of this, all of the compounds tested showed some response either > 10% or < -10% at some concentration level.

The only warfare agent available for testing was β Aflatoxin. It showed a strong negative inhibition at low concentrations but drifted to positive at higher concentrations. This is probably due to its use as a food source. While nerve agents were not available, a surrogate, Ethoprophos, was tested. See figure 1. The results showed that this toxin could be detected at levels as low as 0.01 ppm. Similar results could be expected for VX nerve agent.

Conclusion:

The new toxicity-monitoring tool described in this paper has been shown to be effective at detecting a wide variety of possible chemical threat agents. The test has been shown to be capable of exhibiting a response from each category of agent tested, and all agents except one, Carbaryl, showed a response at one concentration or another. The test is extremely effective at detecting very low doses of some contaminants such as heavy metals, radionuclides, and cyanide.

The fact that the test can be run on any spectrophotometer or colorimeter capable of measuring absorbance at around 600 nm means that most facilities will have no startup instrumentation costs when adopting this method. Most water treatment facilities already posses an instrument of this type. Luminescent based systems would require the purchase of a luminometer, which is an expensive instrument. The ability to use the test on a portable colorimeter or using a color matching system, such as a color disc, combined with the rapid time to results makes the system useful for first responders doing spot checks of water quality. The procedure is not complicated and can be easily mastered by users with a minimum of technical experience. Reagent costs are minimal at around $2 per test.

An additional advantage to this method, especially for small cash strapped municipalities, is that it can be used for more than one purpose. The original concept design for this test was as a tool to monitor wastewater influent to prevent disruptions to a wastewater treatment system. This use becomes even more important as the possibility of terrorists striking a waste treatment system to effect drinking water intakes down stream becomes a possibility. It can also serve a role as an investigative tool to help track pollution plumes to their source and as an initial monitoring tool to reduce costs when whole effluent toxicity monitoring (WET testing) becomes necessary.

The proposed tests simplicity, versatility and its low cost for instrumentation compared to other methods of toxicity monitoring should make it a valuable tool in the arsenal of solutions we deploy to help safeguard our Nation's drinking water supplies.

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